

# Causal Effect of Plasminogen Activator Inhibitor Type 1 on Coronary Heart Disease

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**Background**—Plasminogen activator inhibitor type 1 (PAI-1) plays an essential role in the fibrinolysis system and thrombosis. Population studies have reported that blood PAI-1 levels are associated with increased risk of coronary heart disease (CHD). However, it is unclear whether the association reflects a causal influence of PAI-1 on CHD risk.

**Methods and Results**—To evaluate the association between PAI-1 and CHD, we applied a 3-step strategy. First, we investigated the observational association between PAI-1 and CHD incidence using a systematic review based on a literature search for PAI-1 and CHD studies. Second, we explored the causal association between PAI-1 and CHD using a Mendelian randomization approach using summary statistics from large genome-wide association studies. Finally, we explored the causal effect of PAI-1 on cardiovascular risk factors including metabolic and subclinical atherosclerosis measures. In the systematic meta-analysis, the highest quantile of blood PAI-1 level was associated with higher CHD risk comparing with the lowest quantile (odds ratio=2.17; 95% CI: 1.53, 3.07) in an age- and sex-adjusted model. The effect size was reduced in studies using a multivariable-adjusted model (odds ratio=1.46; 95% CI: 1.13, 1.88). The Mendelian randomization analyses suggested a causal effect of increased PAI-1 level on CHD risk (odds ratio=1.22 per unit increase of log-transformed PAI-1; 95% CI: 1.01, 1.47). In addition, we also detected a causal effect of PAI-1 on elevating blood glucose and high-density lipoprotein cholesterol.

**Conclusions**—Our study indicates a causal effect of elevated PAI-1 level on CHD risk, which may be mediated by glucose dysfunction. (*J Am Heart Assoc.* 2017;6:e004918. DOI: 10.1161/JAHA.116.004918.)

**Key Words:** coronary heart disease • genome-wide association study • Mendelian randomization • plasminogen activator inhibitor type 1 • single nucleotide polymorphism

Plasminogen activator inhibitor type 1 (PAI-1) is the major inhibitor of the fibrinolytic system. It inhibits the effect of plasminogen activators, thereby inhibiting plasmin

formation and downregulating breakdown of fibrin clots. PAI-1 deficiency caused by mutations has been reported to lead to a moderate bleeding disorder.<sup>1</sup> On the flip side, high levels of PAI-1 were reported in some families with thrombophilia.<sup>2,3</sup> Blood PAI-1 antigen levels and activity are strongly correlated and both measures have been used to study the role of PAI-1 in cardiovascular disease. Elevated PAI-1 levels have been observed to be associated with reinfarction and coronary heart disease (CHD) generally.<sup>4</sup> Furthermore, circumventing PAI-1 actions by administering tissue plasminogen activator, one of the key targets inhibited by PAI-1, is an important treatment for acute ischemic stroke.<sup>5</sup>

However, the relationship between PAI-1 and early atherosclerosis and incident CHD remains unclear. Previous studies reported the correlation of PAI-1 with multiple conventional risk factors of CHD, eg, obesity, glycemic traits, and type 2 diabetes mellitus,<sup>6</sup> metabolic syndrome,<sup>7</sup> as well as correlation with vessel wall thickness.<sup>8</sup> In addition, higher PAI-1 expression was observed in coronary artery tissues in the presence of atherogenic lesions.<sup>9,10</sup> These findings raise interest in whether PAI-1 plays a role in early atherosclerosis

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Accompanying Data S1 and Tables S1 through S3 are available at <http://jaha.ahajournals.org/content/6/6/e004918/DC1/embed/inline-supplementary-material-1.pdf>

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versus acute thrombosis. The association of elevated plasma PAI-1 levels with CHD incidence has been reported in longitudinal studies.<sup>11–19</sup> However, this association did not always remain consistent after adjusting for cardiovascular risk factors.<sup>11–14,16–23</sup> On the one hand, these inconsistencies could be due to small sample sizes and/or restricted study populations (eg, type 2 diabetes mellitus patients, obese individuals, or HIV patients).<sup>20,24</sup> On the other hand, the original observational associations including the link between PAI-1 and CHD are potentially prone to bias from unmeasured confounders or overadjustment for mediators.

To overcome these obstacles, epidemiological studies have adapted instrumental variable (IV) analysis to assess causality and to limit confounding through the use of single nucleotide polymorphisms (SNPs) as IV. This method is referred to as the Mendelian randomization (MR) approach.<sup>25</sup> Given that genotypes are assigned randomly from parents to offspring during meiosis, the causal effect of PAI-1 on CHD risk can be estimated by the ratio of a SNP(IV)-PAI-1 association to SNP(IV)-CHD association.<sup>25,26</sup> Using the MR method, a previous study suggested a causal association of PAI-1 with myocardial infarction and blood triglycerides.<sup>27</sup> However, in that study, the association of the SNP, the “4G/5G” polymorphism (rs1799889) in *SERPINE1*, with PAI-1 and CHD risk was based on a meta-analysis using published candidate gene studies between 1993 and 2010.<sup>27</sup> Those observations could be influenced by publication bias, small sample sizes and testing of a single SNP IV. With the recent advent of large-scale genetic studies, an updated view of potential causal associations between PAI-1, CHD, and its risk factors is warranted, and should have better power to untangle potential causal pathways.

In the largest genome-wide association study (GWAS) for PAI-1 (n=19 599 individuals of European ancestry), the CHARGE Hemostatic Working Group reported 4 independent genetic variants from 3 loci (chr7q22.1, chr11p15.2, chr3p25.2).<sup>28</sup> The strongest finding in the study was the *SERPINE1* gene locus, the coding gene region of PAI-1, on chr7q22.1. The lead SNP rs2227631 is in the promoter region of *SERPINE1* and highly correlated with the well-characterized functional variant 4G/5G *SERPINE1* polymorphism ( $r^2=0.78$ ). Following conditional analysis for the lead SNP, a second independent signal (rs6976053) in the same chr7q22.1 locus was observed 200 kb upstream of rs2227631.<sup>28</sup> In total, the genetic variants from the 3 identified loci explained 0.9% variation of plasma PAI-1 levels in the Framingham Heart Study.<sup>28</sup> This strength of IV is in the range of others that have been employed in successful MR studies, suggesting these variants could serve as a potential IV in MR analyses with risk factor, subclinical, and clinical outcomes.<sup>26,29,30</sup>

In this investigation, we aimed to understand whether plasma PAI-1 levels played a causal role in CHD risk. To achieve the goal, we first investigated the observational

association between PAI-1 and CHD using a systematic meta-analysis. We then explored the causal effect of PAI-1 on CHD using a MR approach. Finally, we further investigated the causal effect of PAI-1 on known cardiovascular risk factors, including metabolic risk factors (ie, type 2 diabetes mellitus, body mass index [BMI], waist-hip ratio, fasting blood glucose, insulin and lipids, and blood pressure) and subclinical atherosclerosis measures (ie, carotid intima-media thickness, carotid plaque volume, and coronary artery calcification).

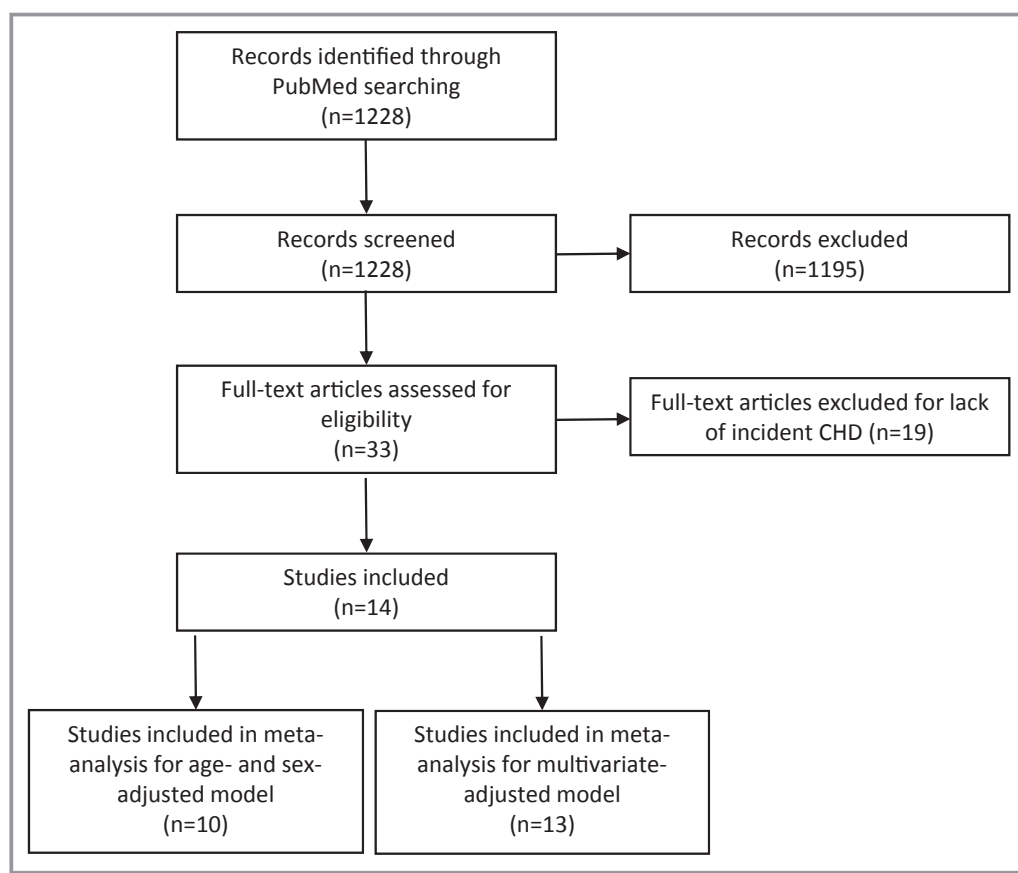
## Methods

### Systematic Meta-Analysis for Observational Association

We applied a systematic review to understand the observational association of PAI-1 with CHD. An electronic literature search was conducted in PubMed by 2 researchers independently using the following criteria: (1) including “Coronary heart disease” or “Coronary artery disease” or “Myocardial infarction”; (2) including “plasminogen activator inhibitor type 1”; (3) published in English from January 1992 to April 2016; and (4) study in human subjects. Two reviewers independently performed the literature screen and found consistent results. In total, we found 1228 articles available in PubMed. There were 33 publications that reported an effect size of PAI-1 on CHD. To focus on association between PAI-1 and incident CHD, we excluded those studies that reported prevalent CHD (13 publications), recurrent CHD (5 publications), or stroke (1 publication). In addition, considering that the majority of publications reported the relative risk of CHD comparing the highest (ie, tertile, quartile, or quintile) with the lowest quantile, we included only publications using categorical analyses of PAI-1. Ten studies that adjusted only for age and sex reported association between PAI-1 and CHD incidence (Table S1), while 13 studies found the same association following adjustment for multiple covariates (eg, BMI, blood glucose, blood lipids, and blood pressure; Table S1).<sup>11–23,31</sup> Covariates used in each study that included multivariable-adjusted models are shown in Table S1. Detailed literature screening procedure is presented in Figure 1. A random-effect meta-analysis was applied in each group of studies using the “metan” package in STATA 13.1.

### Instrumental Variable Analysis for Causal Association

A genetic variant acts as an IV if it fulfils the following assumptions: (1) the genetic variant is associated with the exposure; and (2) the genetic variant can only influence the outcome through the exposure.<sup>32</sup> Burgess et al reported that MR can be applied for causal association using summary



**Figure 1.** Flow chart for article selection in systematic meta-analysis. CHD indicates coronary heart disease.

genetic statistics, ie, beta coefficients with standard errors from genetic association studies.<sup>25</sup> An MR approach using summary data can take advantage of the statistical power of large sample sizes of previous GWASs and does not require multiple phenotypes to be measured in the same study sample. We conducted a power calculation (<https://sb452.shinyapps.io/power/>) for a PAI-1 IV (explaining 0.9% of variance).<sup>28</sup> The results suggest we have 80% statistical power to find a causal odds ratio (OR) larger than 1.15. Thus, we considered the MR analyses viable and obtained summary statistics for circulating PAI-1 levels, CHD and CHD risk factors from previous GWASs as listed in Table 1. These are based on the largest GWAS meta-analysis for each phenotype at the time of analysis and primarily conducted on European ancestry samples.<sup>28,33–42</sup>

We applied 2 sets of genetic variants as IVs. First, we selected multiple genetic variants from the PAI-1 locus chr7q22.1 (*SERPINE1*). In this step, we selected SNPs in this locus that were associated with PAI-1 ( $P < 1 \times 10^{-6}$ ) and that were only moderately correlated with each other after iterative stepwise selection ( $r^2 < 0.5$  each round). The correlations between SNPs were obtained from the bioinformatics tool SNiPA using data from the 1000 Genomes phase 3,

European reference population.<sup>43</sup> This resulted in 4 selected SNPs (rs2227631, rs2075756, rs12672665, and rs757718; Table S2). A genetic risk score as IV was then constructed by adding the number of risk alleles and weighting each risk allele dose by its effect on PAI-1. We further corrected for the correlation between each SNP in this genetic risk score in our IV analysis following the method developed by Burgess et al.<sup>44</sup> In the second step, we extended the genetic risk score by using multiple loci from the PAI-1 GWAS that were shown to reach genome-wide significance ( $P < 5 \times 10^{-8}$ ). Four independent SNPs reported from the PAI-1 GWAS were used in the IV (rs2227631, rs6976053, rs6486122, and rs11128603; Table S2),<sup>28</sup> and then a genetic risk score was generated as IV by adding the counts of risk alleles weighted by their effects on PAI-1. The second IV was only applied for the causal association between PAI-1 and CHD, but not between PAI-1 and cardiovascular risk factors.

The estimates for associations of SNPs with PAI-1 are reported per risk allele change of units of log-transformed PAI-1.<sup>28</sup> The estimates for associations of SNPs with CHD are reported per risk allele change of CHD risk.<sup>39</sup> The causal effect of a per unit change of log-transformed PAI-1 on CHD is estimated as the per risk allele change of CHD risk dependent

**Table 1.** List of Genome-Wide Association Studies Used in the Current Study

| Trait   | Consortium        | Sample Sizes                | Unit              | Transformation                 | Reference                     |
|---|-------------------|-----------------------------|-------------------|--------------------------------|-------------------------------|
| SNP association with the exposure   |                   |                             |                   |                                |                               |
| PAI-1   | CHARGE            | 19 599                      | ng/mL             | log-transformed                | Huang et al <sup>28</sup>     |
| SNP association with the primary outcome                                    |                   |                             |                   |                                |                               |
| CHD*  | CARDIOGRAMplusC4D | 60 801/123 504 <sup>†</sup> | Case/control      | N/A                            | Nikpay et al <sup>39</sup>    |
| SNP association with the potential intermediators via metabolic syndrome    |                   |                             |                   |                                |                               |
| Type 2 diabetes mellitus  | DIAGRAM           | 34 840/114 981 <sup>†</sup> | Case/control      | N/A                            | Morris et al <sup>38</sup>    |
| Fasting blood glucose   | MAGIC             | 58 074                      | mmol/L            | N/A                            | Manning et al <sup>37</sup>   |
| Fasting blood insulin   | MAGIC             | 51 750                      | pmol/L            | Log-transformed                | Manning et al <sup>37</sup>   |
| Blood lipids <sup>‡</sup>   | GLGC              | 188 577                     | mmol/L            | Quantile normalization         | Willer et al <sup>42</sup>    |
| Blood pressure <sup>§</sup>   | ICBP              | 203 056                     | mm Hg             | Inverse standard normalization | Ehret et al <sup>35</sup>     |
| BMI   | GIANT             | 339 224                     | kg/m <sup>2</sup> | Inverse standard normalization | Locke et al <sup>36</sup>     |
| Waist-hip ratio   | GIANT             | 224 459                     | cm/cm             | Inverse standard normalization | Shungin et al <sup>41</sup>   |
| Adiponectin   | ADIPOGen          | 39 883                      | μg/mL             | Log-transformed                | Dastani et al <sup>34</sup>   |
| SNP association with the potential intermediators via early atherosclerosis |                   |                             |                   |                                |                               |
| IMT   | CHARGE            | 31 211                      | mm                | Log-transformed                | Bis et al <sup>33</sup>       |
| Carotid plaque <sup>  </sup>  | CHARGE            | 12 955/18 263 <sup>†</sup>  | Case/control      | N/A                            | Bis et al <sup>33</sup>       |
| CAC   | CHARGE            | 9961                        | Agatston score    | Log-transformed                | O'Donnell et al <sup>40</sup> |

BMI indicates body mass index; CAC, coronary artery calcification; CHD, coronary heart disease; IMT, intima-media thickness; N/A, not applicable; PAI-1, plasminogen activator type 1; SNP, single nucleotide polymorphism.

\*CHD cases include myocardial infarction and unstable angina.

<sup>†</sup>Sample sizes of CHD, type 2 diabetes mellitus, and plaque are split into cases and controls.

<sup>‡</sup>Blood lipids includes serum total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides.

<sup>§</sup>Blood pressure includes systolic blood pressure and diastolic blood pressure measures.

<sup>||</sup>Plaque cases are individuals with presence of carotid plaque.

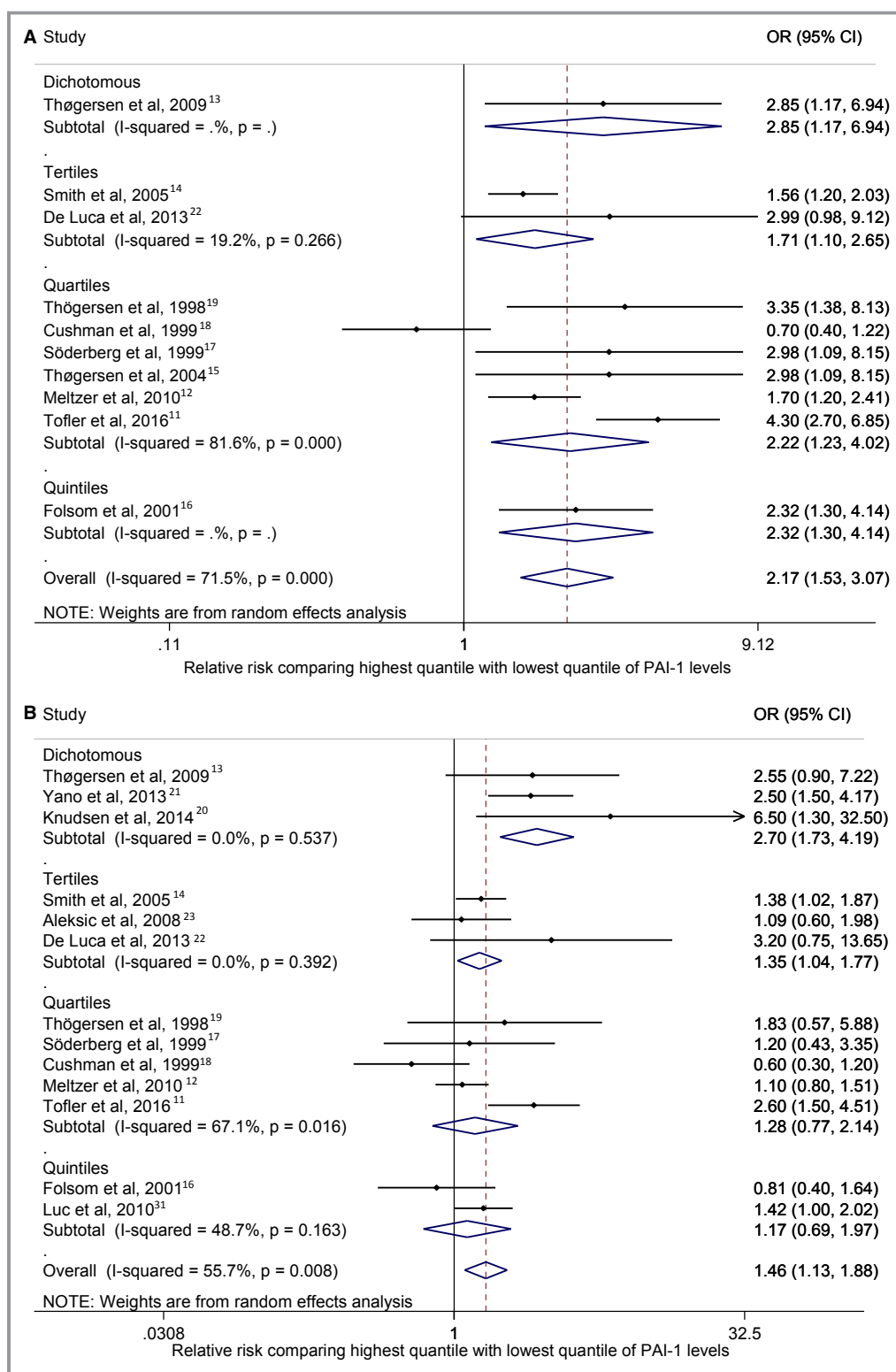
on the per risk allele change of log-transformed PAI-1 units. When using 1 SNP as an IV, it is calculated as the ratio estimate of the SNP-CHD association to the SNP-PAI-1, that is,  $\beta_{\text{SNP-CHD association}} / \beta_{\text{SNP-PAI-1 association}}$ .<sup>26</sup> When using multiple SNPs as an IV, the combined causal effect was evaluated as the inverse-variance weighted estimate of the causal ratio when using each SNP alone as an IV.<sup>25</sup> The 95% CIs for causal estimates are calculated based on the estimates (beta/log-transformed OR) and SE: estimate  $\pm 1.96 \times \text{SE}$ . The IV analysis was performed using R version 3.1.2.

## Results

In the systematic review, most previous studies reported PAI-1 and CHD incidence association based on PAI-1 levels in quartiles, while there were 3 studies based on dichotomizing PAI-1 levels, 3 using tertiles and 2 using quintiles. A pooled meta-analysis shows the highest quantile (ie, tertile, quartile, or quintile) of blood PAI-1 levels is associated with higher risk of CHD incidence compared with the lowest quantile (OR=2.17; 95% CI: 1.53, 3.07; Figure 2A) in an age- and sex-adjusted model.<sup>11–19,22</sup> The overall heterogeneity across

studies is high ( $I^2=71.5\%$ ,  $P<0.001$ ; Figure 2A). The association estimate is reduced but remains significant in studies using a multivariable-adjusted model (OR=1.46; 95% CI: 1.13, 1.88; Figure 2B),<sup>11–14,16–23,31</sup> with a lower overall heterogeneity compared with studies applying only an age- and sex-adjusted model ( $I^2=55.7\%$ ,  $P=0.008$ ; Figure 2B). In the subgroup meta-analysis based on different PAI-1 quantile scales, heterogeneity is observed most strongly in the quartile subgroup in both age- and sex-adjusted model ( $I^2=81.6\%$ ,  $P<0.001$ ; Figure 2A) and the multivariable-adjusted model ( $I^2=61.7\%$ ,  $P<0.016$ ; Figure 2B).

Using variants in the *SERPINE1* locus as IVs, the MR analysis suggests that, under the assumptions of the MR approach, an increase of one unit of log-transformed PAI-1 level can increase CHD risk by 22% (OR=1.22; 95% CI: 1.02, 1.45; Table 2). When variants in multiple loci are used as IVs, the result is consistent and the confidence interval narrows slightly (OR=1.25; 95% CI: 1.07, 1.45). We constructed scatter plots for genetic associations with CHD against genetic associations with PAI-1 for the 2 sets of SNPs as IV separately (Figure 3). Figure 3A shows that all SNPs from the *SERPINE1* locus are associated with PAI-1 and CHD in a



**Figure 2.** Observational associations of PAI-1 and CHD from the literature up until July 2016. This is a forest plot depicting the result of the meta-analysis based on previous publications for observations of plasminogen activator inhibitor type 1 (PAI-1) and coronary heart disease (CHD) association. Odds ratio (OR) with 95% CI is the OR of CHD comparing the highest PAI-1 quantile to lowest PAI-1 quantile, which is also represented as a point with bar in the plot. The diamond represents the meta-analysis result using a random effects model. A, PAI-1-CHD associations adjusted for age, sex, and ethnic group in each study. B, PAI-1-CHD association in models adjusted for multiple CHD risk factors.



**Table 2.** Causal Effect of PAI-1 on Cardiovascular Risk Factors Using the *SERPINE1* Locus as IV

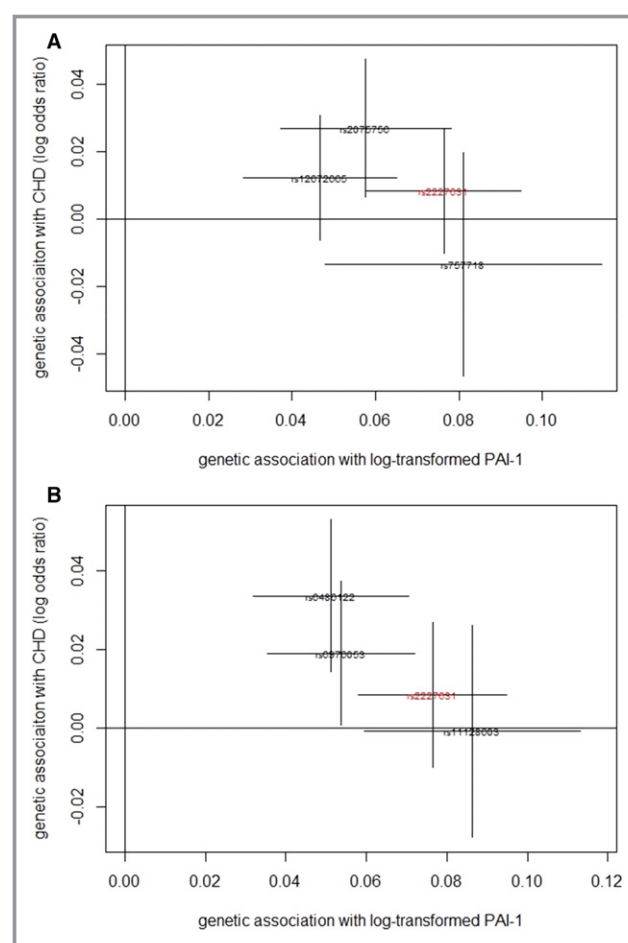
| Trait                       | Effect | 95% CI        | P Value |
|-----------------------------|--------|---------------|---------|
| CHD*                        | 1.22   | (1.01, 1.47)  | 0.039   |
| Metabolic risk factors      |        |               |         |
| Type 2 diabetes mellitus*   | 1.18   | (0.85, 1.62)  | 0.321   |
| Fasting blood glucose       | 0.08   | (0.02, 0.14)  | 0.012   |
| Fasting blood insulin       | −0.002 | (−0.07, 0.06) | 0.939   |
| Total cholesterol           | 0.08   | (−0.02, 0.19) | 0.113   |
| HDL-C                       | 0.13   | (0.04, 0.23)  | 0.008   |
| LDL-C                       | 0.03   | (−0.08, 0.13) | 0.583   |
| Triglycerides               | −0.03  | (−0.12, 0.23) | 0.578   |
| BMI                         | −0.07  | (−0.14, 0.01) | 0.070   |
| Waist-hip ratio             | −0.07  | (−0.15, 0.02) | 0.112   |
| Systolic blood pressure     | 1.22   | (−0.77, 3.20) | 0.230   |
| Diastolic blood pressure    | 0.20   | (−1.06, 1.46) | 0.758   |
| Adiponectin                 | 0.004  | (−0.08, 0.09) | 0.926   |
| Subclinical atherosclerosis |        |               |         |
| IMT                         | 0.01   | (−0.02, 0.04) | 0.669   |
| Carotid plaque*             | 1.03   | (0.98, 1.58)  | 0.876   |
| CAC                         | 0.33   | (−0.29, 0.95) | 0.293   |

BMI indicates body mass index; CAC, coronary artery calcification; CHD, coronary heart disease; HDL-C, high-density lipoprotein cholesterol; IMT, intima-media thickness; IV, instrumental variable; LDL-C, low-density lipoprotein cholesterol; PAI-1, plasminogen activator type 1.

\*The traits with marked with \* are dichotomous traits and effects/95% CI for these traits were reported as odds ratio. Other traits are all continuous traits, and their effects/95% CI were reported as beta coefficients.

similar manner. That is, when one SNP has a larger effect size on PAI-1, it also has a relatively larger effect size on CHD risk. This suggests that the results using the combined genetic risk score in set 1 (*SERPINE1* locus) as IV are not simply driven by a single SNP. Similarly, Figure 3B suggests that the results when using multiple loci in the IV are not driven by a single SNP.

MR analyses for causal effects of PAI-1 on cardiovascular risk factors using variants in the *SERPINE1* locus alone suggest that an increase of 1 unit of log-transformed PAI-1 level increases circulating fasting glucose levels by 0.08 mmol/L ( $\beta=0.08$ ; 95% CI: 0.02, 0.14; Table 2); and increases high-density lipoprotein cholesterol (HDL-C) by 0.13 SDs ( $\beta=0.13$ ; 95% CI: 0.04, 0.23; Table 2). We found no evidence for causal effects of PAI-1 on other metabolic risk factors, or subclinical atherosclerosis. However, the result for BMI suggests a negative effect of PAI-1 on BMI with a trend toward significance ( $\beta=-0.07$ ,  $P=0.070$ ; Table 2), which contradicts most epidemiological studies demonstrating a positive association.<sup>45,46</sup> Therefore, we further investigated the causal effect of BMI on PAI-1 levels using 77 genome-wide



**Figure 3.** Scatter plots for genetic associations with CHD against genetic associations with PAI-1. This shows scatter plots of genetic associations with coronary heart disease (CHD) against genetic associations with plasminogen activator inhibitor type 1 (PAI-1). Lines in the horizontal and vertical directions represent 95% CI of genetic associations with PAI-1 and CHD, respectively. The SNP marked in red (rs2227631) located in *SERPINE1* is the SNP with the lowest  $P$ -value reported in the genome-wide association study of PAI-1.<sup>28</sup> A, Scatter plot of 4 correlated SNPs selected in the *SERPINE1* locus as instrumental variable. B, Scatter plot of 4 independent SNPs selected from multiple loci as instrumental variable. SNP indicates single nucleotide polymorphism.

significant loci identified in a large BMI GWAS in European populations (Data S1).<sup>36</sup> This result shows that BMI has a causal effect on PAI-1 in the positive direction ( $\beta: 0.21$ ; 95% CI: 0.13, 0.29; Table S3), and was consistent with sensitivity analyses using a median estimator approach and MR-Egger regression to test for potential pleiotropic effects.

## Discussion

The systematic meta-analysis using the available epidemiological literature supports the association between PAI-1 and

CHD incidence, independent of established cardiovascular risk factors. Given the heterogeneity across studies, we further utilized the MR approach. This approach has been successful in supporting (low-density lipoprotein cholesterol) and refuting (HDL-C) causal links to CHD that mirror clinical trial results.<sup>47,48</sup> The results of our MR study do support a causal link between PAI-1 and CHD. In addition, the MR analyses also suggest a casual effect of PAI-1 levels on blood glucose levels and HDL-C levels. Our study represents a comprehensive investigation of the effect of PAI-1 on CHD and its risk factors in well-powered population samples, suggesting potential mechanisms for further investigation or potential intervention. We investigated the association between PAI-1 and incident CHD via systematic meta-analysis using current publications. Additionally, this study is the first to report the causality of PAI-1 on CHD and CHD risk factors using GWAS summary statistics. By leveraging large sample sizes reaching over 60 000 cases and 120 000 controls from GWAS consortia, we find a robust causal association of PAI-1 with CHD.

A key assumption for the MR approach is that genetic variants employed as the IV can only be associated with the outcome (CHD) through the biomarker (PAI-1). The causal effect of PAI-1 on CHD suggested by the MR approach should be interpreted under this assumption. In addition, further functional studies are required to understand the mechanism of the causal association between PAI-1 and CHD. As a protein biomarker, the genetic locus encoding the PAI-1 transcript has clear biological function in determining circulating PAI-1 levels. The 4G/5G polymorphism in the promoter region of *SERPINE1* has been consistently reported to be a functional variant influencing PAI-1 expression.<sup>27,49</sup> Knockout of *Serpine1*, a mouse ortholog, creates PAI-1 deficiency.<sup>50</sup> Therefore, when exploring whether metabolic risk factors and subclinical atherosclerosis are mediators of potential PAI-1 effects on CHD, we only used the *SERPINE1* locus SNPs as an IV.

Our study is the first evidence to suggest a causal association of PAI-1 on increased fasting glucose. This indicates PAI-1 may play a role in glucose regulation and is consistent with previous population studies that reported positive correlations between circulating PAI-1 and glucose levels.<sup>6,51,52</sup> In addition to observational studies, experimental studies showed that PAI-1 deficiency via genetic knock-out or pharmacological inhibition can suppress the levels of blood glucose in mice.<sup>53,54</sup> MR analysis of PAI-1 with type 2 diabetes mellitus had a consistent effect direction with what is expected based on the glucose findings, but was not significant (Table 2). This finding potentially suggests a causal pathway of PAI-1 to CHD risk, mediated by elevated glucose level. However, a mediation test would be required to verify this conclusion using individual-level data with genetics, PAI-1 levels, fasting glucose, and CHD in the same study population.

Somewhat surprisingly, when addressing the causal effect of PAI-1 on measurements of obesity, we find negative trend effects of PAI-1 on BMI and waist–hip ratio. Adipose tissue is one of the main tissues expressing PAI-1, and population studies have consistently shown positive correlations between circulating PAI-1 levels and BMI.<sup>51</sup> Ex vivo studies suggested the bidirectional regulation between PAI-1 and adipocytes. For example, Crandall et al suggested that endogenous expression of PAI-1 might regulate adipogenesis by preventing preadipocyte migration into cell clusters.<sup>55</sup> Halleux et al reported that the expression of PAI-1 in cultured human adipose tissue elevated in response to glucocorticoids.<sup>56</sup> Therefore, we conducted analysis on the causal effect of BMI on PAI-1 levels. The results indicated a potential positive causal effect of BMI on PAI-1. Taken together, we conclude that the association between circulating PAI-1 levels and BMI may be due to a causal effect of BMI on PAI-1 rather than PAI-1 regulation on BMI.

Our results suggest a further positive causal effect of PAI-1 on HDL-C, which is inconsistent with observational associations in the population study.<sup>51</sup> However, an HDL-C influence on CHD is itself paradoxical. While observational studies consistently report a protective association of high blood HDL-C levels with lower CHD risk, previous MR studies show that HDL-C is not a causal risk factor for CHD.<sup>48,57</sup> Furthermore, a recent study has reported that a loss-of-function variant in scavenger receptor BI (*SRB1*) raises HDL-C and increases CHD risk.<sup>58</sup> Further studies are warranted to understand potential biological mechanisms of a PAI-1 effect on HDL-C and whether HDL-C is an intermediary between the PAI-1 and CHD associations.

There are several limitations of the current study. Since we used summary GWAS statistics in the current study, we were unable to address stratified analysis questions such as whether there is a sex or age difference in the PAI-1-CHD link, or whether the effect of PAI-1 on CHD differs among obese individuals versus nonobese individuals. These are interesting questions for future studies. In addition, our reported observational meta-analysis between PAI-1 and CHD is based on PAI-1 quantiles, while the causal association is based on log-transformed PAI-1 units; therefore, the effect size of PAI-1 on CHD in these 2 sets of analysis is not directly comparable.

In summary, we applied several approaches to understand the role of PAI-1 in CHD. Our results through several analyses support a causal effect of PAI-1 on CHD onset, potentially mediated by blood glucose dysfunction. Furthermore, our results and those of others suggest that PAI-1 may be interlocked with obesity, and potentially HDL-C in complex feedback relationships. Our study adds to evidence on the role of PAI-1 in pathogenesis of CHD and suggests this pathway may be a good target for CHD treatment.

## Appendix

### CHARGE Consortium Hemostatic Factor Working Group

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## Data S1

### Methods for causal effect of BMI on PAI-1

To further investigate our findings on the negative direction of effect of PAI-1 on BMI, which runs counter to some prior evidence,<sup>1</sup> we explored the causal effect of BMI on PAI-1. Locke *et al.* reported 77 independent genome-wide significant loci associated with BMI in 322,154 individuals of European ancestry.<sup>2</sup> We applied the MR approach using all 77 loci as an instrumental variable (IV) using the inverse variance weighted (IVW) method described in our main article. Two sensitivity analyses were further adopted to examine potential pleiotropic effects of the selected IV. In the first sensitivity analysis, we used the MR-Egger method.<sup>3</sup> In this method, an Egger regression, which is commonly used to detect small study bias, was used to detect the potential bias of pleiotropic effects in the MR. The beta coefficient in the MR-Egger is considered as a causal effect after correcting for pleiotropic effects. The intercept of the Egger regression provides information on the directional pleiotropic effect in the IV (right panel in **Table S3**).<sup>3</sup> In the second sensitivity analysis, we investigated whether the causal effect was consistent when only 50% of the SNPs are assumed to be valid in the IV, an approach known as weighted median estimator.<sup>4</sup>

### Results for causal effect of BMI on PAI-1

We found that increasing 1 unit of BMI was causally associated with a 0.21 unit increase of log-transformed PAI-1 (beta, 0.21, 95% CI, 0.13, 0.29; **Table S3**). The result was consistent when the MR-Egger method was applied (beta, 0.21, 95% CI, 0.02, 0.41; **Table S3**), with little influence of pleiotropic effect as indicated by the intercept (beta, -0.0002, P-value, 0.949). The median estimator was also in agreement with the other two analyses (beta, 0.22, 95% CI, 0.09, 0.36; **Table S3**). Taken together, our results support a robust positive causal effect of BMI on PAI-1.

**Table S1. Publications included in the observational meta-analysis**

| Study                                       | PMID     | Adjustment in the multiple-variable model   |
|---|----------|---|
| Thøgersen <i>et al</i> , 1998 <sup>5</sup>  | 9826309  | Age, sex, diabetes, smoking, hypertension, BMI, Cholesterol, and ApoA-1.  |
| Cushman <i>et al</i> , 1999 <sup>6</sup>    | 10073948 | Hypertension, smoking status, race (white or nonwhite), diabetes, and body mass index.  |
| Söderberg <i>et al</i> , 1999 <sup>7</sup>  | 10583712 | BMI, hypertension, history of diabetes, daily smoking habits, cholesterol levels, leptin, apo A-1, apo B, and insulin.  |
| Folsom <i>et al</i> , 2001 <sup>8</sup>     | 11304480 | Age, race, and sex, smoking status (never, former, current), total cholesterol, HDL cholesterol, systolic blood pressure, use of antihypertensive medication, and diabetes. |
| Thøgersen <i>et al</i> , 2004 <sup>9</sup>  | 15167204 | N/A (dichotomous analysis).   |
| Smith <i>et al</i> , 2005 <sup>10</sup>     | 16286603 | Age, sex, race, hypertension, diabetes mellitus, total cholesterol, HDL, cigarette smoking and alcohol intake.  |
| Aleksic <i>et al</i> , 2009 <sup>11</sup>   | 18342864 | Age, sex, race, hypertension, diabetes mellitus, total cholesterol, HDL, cigarette smoking and alcohol intake.  |
| Thøgersen <i>et al</i> , 2009 <sup>12</sup> | 19357504 | Age, smoking, CRP, t-PA, creatinine.  |
| Luc <i>et al</i> , 2010 <sup>13</sup>       | 19823188 | Diabetes, hypertension, smoking status, total and high-density lipoprotein (HDL) cholesterol and triglycerides.   |
| Meltzer <i>et al</i> , 2010 <sup>14</sup>   | 20413657 | Age, HDL and total cholesterol, triglycerides, BMI, and diabetes.   |
| Yano <i>et al</i> , 2013 <sup>15</sup>      | 23551722 | Sex, body mass index, history of diabetes, history of hyperlipidemia, and 24-hour pulse rate, high levels of high-sensitivity C-reactive protein, prothrombin fragment 1+2. |
| De Luca <i>et al</i> , 2013 <sup>16</sup>   | 24004495 | Duration of time between the date of samples and the analysis time, age, total cholesterol, HDL.  |

|   |          |  |
|---|----------|--|
| Knudsen <i>et al</i> , 2014 <sup>17</sup> | 24566095 | Viral load and a high (Data collection on Adverse events of Anti-HIV Drugs) D:A:D risk score.  |
| Tofler <i>et al</i> , 2016 <sup>18</sup>  | 26896607 | Age, sex, systolic blood pressure, anti-hypertensive therapy, BMI, diabetes, cigarette smoking, total cholesterol, HDL cholesterol, and triglycerides. |

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**Table S2. SNPs involved in the genetic risk scores as instrumental variable for PAI-1**

| SNPId   | Chr:position(hg19) | Risk/Other alleles | Allele freq in PAI-1 <sup>*</sup> | Allele freq in CHD <sup>*</sup> | Effect on PAI-1 <sup>**</sup> | Effect on CHD <sup>***</sup> |
|---|--------------------|--------------------|-----------------------------------|---------------------------------|-------------------------------|------------------------------|
| SNPs involved in the single locus instrumental variable |                    |                    |                                   |                                 |                               |                              |
| rs2227631   | chr7:100,769,538   | A/G                | 0.592                             | 0.563                           | 0.076 (0.010)                 | 0.008 (0.009)                |
| rs2075756   | chr7:100,466,441   | A/G                | 0.282                             | 0.279                           | 0.058 (0.010)                 | 0.027 (0.010)                |
| rs12672665  | chr7:100,483,731   | A/G                | 0.479                             | 0.474                           | 0.047 (0.009)                 | 0.012 (0.009)                |
| rs757718  | chr7:100,792,810   | T/C                | 0.093                             | 0.118                           | 0.081 (0.016)                 | -0.013 (0.017)               |
| SNPs involved in the multi locus instrumental variable  |                    |                    |                                   |                                 |                               |                              |
| rs2227631   | chr7:100,769,538   | A/G                | 0.592                             | 0.563                           | 0.076 (0.010)                 | 0.008 (0.009)                |
| rs6976053   | chr7:100,512,119   | T/C                | 0.479                             | 0.476                           | 0.054 (0.009)                 | 0.019 (0.009)                |
| rs6486122   | chr11:13,361,524   | T/C                | 0.689                             | 0.638                           | 0.051 (0.009)                 | 0.034 (0.010)                |
| rs11128603  | chr3:12,385,828    | A/G                | 0.898                             | 0.870                           | 0.086 (0.016)                 | -0.001 (0.014)               |

\* Allele freq in PAI-1 and CHD reported the allele frequency of the risk allele in the study samples for PAI-1 and CHD respectively.

\*\* Effect of SNPs on PAI-1 is reported as beta coefficient (with standard error) from Huang *et al*, 2012.<sup>19</sup>

\*\*\* Effect of SNPs on CHD is reported as log-transformed Odds ratio (with standard error) from Nikpay *et al*, 2015.<sup>20</sup>



**Table S3. Causal effect of BMI on PAI-1**

| Method                             | Causal effect of BMI on PAI-1 |            |          | Directional pleiotropic effect |         |
|------------------------------------|-------------------------------|------------|----------|--------------------------------|---------|
|                                    | Beta                          | 95% CI     | P-value  | Beta                           | P-value |
| <b><i>Primary MR analysis</i></b>  |                               |            |          |                                |         |
| IVW approach                       | 0.21                          | 0.13, 0.29 | 4.31E-07 | N/A                            | N/A     |
| <b><i>Sensitivity analysis</i></b> |                               |            |          |                                |         |
| MR_Egger                           | 0.21                          | 0.02, 0.41 | 0.034    | -0.0002                        | 0.949   |
| Weighted median estimator          | 0.22                          | 0.09, 0.36 | 0.001    | N/A                            | N/A     |

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